IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appl. No. : **10/733,706** Confirmation No. 8647

Applicants : **James E. Hagstrom, et al.**

Filed : **12/11/2003**

Art Unit : **1636**

Examiner : **Kaushal, Sumesh**

Docket No. : **Mirus.048.01**

For: **A Process Of Delivering A Virally Encapsulated Polynucleotide Or Viral Vector To A Parenchymal Cell Via The Vascular System**

Commissioner of Patents

PO Box 1450

Alexandria, VA 2231-1450

AMENDMENT UNDER 37 C.F.R. ' 1.111

Dear Examiner:

I, James E. Hagstrom, hereby declare as follows:

1. I am an inventor of the captioned application.
2. I submit with this Declaration and Response further experimental material (attached) illustrating: delivery of viral vectors to limb extravascular cells without co-administration of papaverine or other vasodilator.
3. The material is consistent with the specification as filed and only methods described in the specification have been used. No new matter was used in the experiments.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

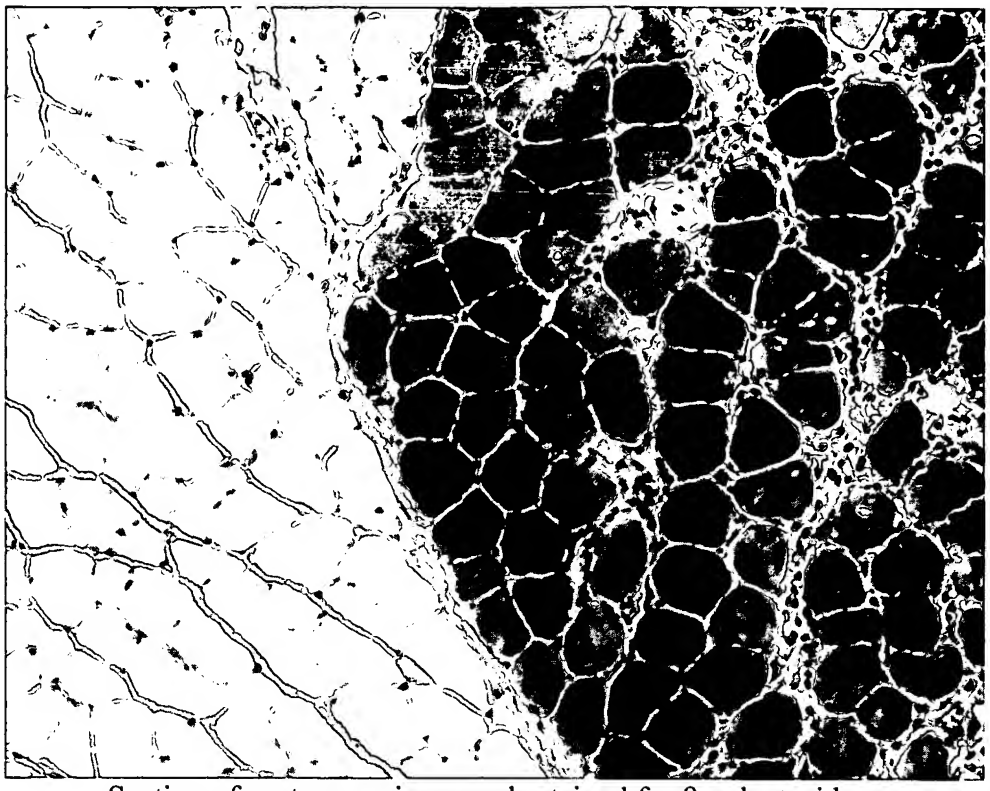
3/14/05

Dr. James E. Hagstrom Date

Delivery of virus to limb extravascular cells

Study #03190401:

Delivery of Adeno-associated virus to mouse limb skeletal muscle cells via injection into limb vein. A male ICR mouse was anesthetized with 2% isoflurane. A latex tourniquet was wrapped tightly around the upper limb and secured with a hemostat. The distal great saphenous vein was exposed through a 1-2 cm incision. A 27 gauge butterfly needle catheter was inserted into the great saphenous vein and connected to a syringe pump (Harvard PHD 2000). For the injection, 0.8 ml of saline containing 0.18×10^{9} rAAV transducing units (CMV-lacZ, Virus Vector Core, Univ North Carolina) was injected at a rate of 3.0 ml/min. Two minutes after the injection the tourniquet and catheter were removed and the incision was closed with suture. The animal was harvested at 2 weeks after the injection.

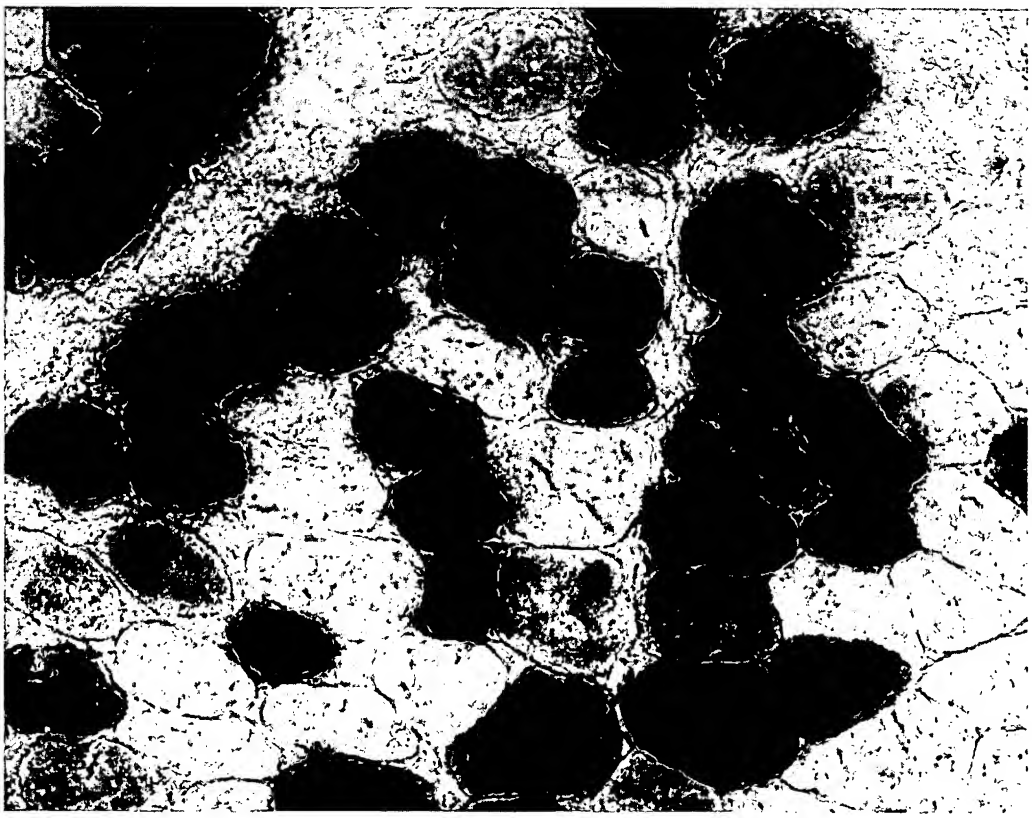


Section of gastrocnemius muscle stained for β-galactosidase

Section of hamstring muscle stained for β-galactosidase

Study # 04160402:

Delivery of Adeno-associated virus to rat limb skeletal muscle cells via injection into limb vein. A female Sprague Hawley rat was anesthetized with 2% isoflurane. A latex tourniquet was wrapped tightly around the upper limb and secured with a hemostat. The distal great saphenous vein was exposed through a 2-3 cm incision. A 25 gauge butterfly needle catheter was inserted into the great saphenous vein and connected to a syringe pump (Harvard PHD 2000). The animal were given a preinjection of 1.5 ml saline followed by 3.0 ml of saline containing 1.875×10^{10} rAAV transducing units (CMV-lacZ, Virus Vector Core, Univ North Carolina). Both injections were done at a rate of 10 ml/min. Two minutes after the injection the tourniquet and catheter were removed and the incision was closed with suture. The animal was harvested at 2 weeks after injection.



Section of gastrocnemius muscle stained for β-galactosidase